Ecological and Geographical Reasons for the Variation of Digestive Tract Length in Anurans

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Abstract Changes of environmental conditions can shape organs size evolution in animal kingdoms. In particular, environmental changes lead to difference in food resources between different habitats, thereby affecting individual's energy intake and allocation. The digestive theory states that animals consuming food with low contents of digestible materials should result in increasing gut length. In this study, to test the hypothesis of digestive theory, we studied ecological and geographical reasons for variation in digestive tract length among 35 species of anurans distributing in different altitude and latitude. The results showed that ecological type significantly affected digestive tract length among species, with aquatic and terrestrial species having longer digestive tract than arboreal ones. Latitude was positively correlated with digestive tract length. However, altitude, as well as monthly mean temperature and precipitation, did not correlate with digestive tract length among species. Our findings suggest that aquatic and terrestrial species might forage less digestible materials than arboreal species, thereby displaying relatively longer digestive tract than arboreal species.

Keywords Anurans, digestive tract length, environmental change, ecological type

1. Introduction

Evolution in size of organ in organisms has caused concerns for evolutionary biologists and ecologists for decades (Piersma and Lilliendahl, 1999; Hammond *et al*., 1999; Liao *et al*., 2016a; Chen *et al*., 2016; Mai *et al*., 2017a,b; Tanner *et al*., 2017; Alton *et al*., 2017; Kotrschal *et al*., 2017; Liao *et al*., 2018; Yang *et al*., 2018; Güneş and Danacıoğlu, 2018; Medini *et al*., 2018; Liu *et al*., 2018; Samuk *et al*., 2018; Cai *et al*., 2019; Zhao *et al*., 2019). In particular, energy store is an important factor affecting variation in the organ size of ectotherms in different environments (Lüpold *et al*., 2017; Signor *et al*., 2017; Hammond *et al*., 2000; Jin *et al*., 2016a,b;

Luo *et al*., 2017; Gu *et al*., 2017; Iwai, 2018; Joseph *et al*., 2018; Huang *et al*., 2018; Tang *et al*., 2018; Mai and Liao, 2019). For instance, to improve local adaptation, individuals living in more biotic conditions require more accumulated energy by increasing liver and muscle mass than individuals living in less biotic environments in anurans (Hesse, 1924; Müller *et al*., 2014; Zhong *et al*., 2017; Yang *et al*., 2017).

The digestive tract length varies with changing environments to meet variations in food availability (Sibly, 1981). Actually, the digestive tract is linked to species adaptations in the process of evolution at different external environments in most vertebrates (Naya and Bozinovic, 2004; Naya *et al*., 2007; Naya *et al*., 2008; Naya *et al*., 2009; Lou *et al*., 2013; Ma *et al*., 2016; Wang *et al*., 2017). Consequently, adaptive radiation of the digestive tract to functional demands could provide for an important energy-saving mechanism.

The digestive theory states that animals consume food with low contents of digestible materials, which results in an increase in gut dimensions (Penry and Jumars, 1987). The prediction of this theory has been successfully examined across a broad range of vertebrate taxa (Hansson, 1985; Secor, 2001; Hansson and Jaarola, 1989; Naya *et al*., 2009; Lou *et al*., 2013; Ma *et al*., 2016).

Ecological factors, such as ecological type, has been identified affecting local adaptation of animals (Gonda *et al*., 2009; Liao *et al*., 2015; Shine, 1989; Liao *et al*., 2013; Zeng *et al*., 2014; Liao *et al*., 2014; Liao *et al*., 2016b; Huang *et al*., 2019). For instance, digestive tract variation is confirmed to be correlated with habitat differences of species experienced (Nuñez *et al*., 1982). Anurans live in broad range of different habitats (Fei *et al*., 2010; Liao *et al*., 2018) and the patterns of digestive tract variation associated with ecological type have been less studied than that of other animal groups (Hansson, 1985; Hansson and Jaarola, 1989; Sassi *et al*., 2007; Naya *et al*., 2008). Only recently studies on the intra-specific variation in digestive tract length in anurans have elucidated the influence of seasonality and geographical location (Naya *et al*., 2009; Lou *et al*., 2013; Ma *et al*., 2016; Wang *et al*., 2017). However, studies on the inter-specific variation in digestive tract length associated with ecological and geographical reasons among anurans are lacking. Here, we investigated the effects of ecological type on variation in digestive tract length across 35 species of anurans. We also investigated the effects of geographical location (e.g., altitude, latitude, temperature and rainfall) on digestive tract length.

2. Materials and Methods

2.1. Data collection A total of 328 adult males from 35 species were collected by hand at night using a flashlight during the breeding season between 2007 and 2018 from Hengduan Mountains in China (Table S1). All individuals then were taken back to lab and kept singly in wire-netting rectangular containers (20 cm × 10 cm × 15 cm; L × W × H) placed in a tank (90 cm × 40 cm × 40 cm; L × W × H) with a depth of 10 cm of fresh water. All individuals were killed by single- or double-pithing (Yu *et al*., 2018) and preserved in 4% phosphate buffered formalin for tissue fixation. After two to eight weeks of preservation, body size (snout-vent length: SVL) was measured to the nearest 0.01 mm with a caliper, and body mass was weighed to the nearest 0.1 mg with an electronic balance (Wu *et al*., 2016; Zeng *et al*., 2016). Once the connecting mesenteries were cut, the entire

digestive tract was aligned along a caliper with stretching it (Lou *et al*., 2013). We measured digestive tract length to the nearest 0.01 mm with a caliper.

We classified ecological type for each species on a four-point scale: 1 = arboreal – occur mostly on trees, forage in trees and rarely come down to the ground; 2 = terrestrial – occur and forage mostly on ground; 3 = aquatic – semiaquatic – not entirely aquatic, uses both aquatic and terrestrial habitats; and 4 = aquatic – occur and forage mostly in water.

2.2. Phylogeny We constructed the new molecular phylogeny based on a matrix of three nuclear and three mitochondrial genes with good coverage across our 35 species (≥ 13 species for each gene). The mitochondrial gene consisted of the large and small subunits of the mitochondrial ribosome genes (12S/16S) and the cytochrome b (CYTB). The nuclear genes consisted of the recombination-activating gene 1 (RAG1), the tyrosinase (TYR) and the rhodopsin (RHOD). We provided genbank accession numbers for the gene sequences used to generate the phylogeny (Table S2). The sequences were aligned using the MUSCLE function in MEGA v.6.0.6 (Tamura *et al*., 2013) and each gene of the best nucleotide substitution model was determined using the Akaike Information Criterion in jModelTest v.2.1.2 (Darriba *et al*., 2012). The best substitution model was GTR+G for 12S and TYR, HKY+G for RAG1 and RHOD, and GTR+I+G for CYTB and 16S, respectively.

Using these models, we constructed the phylogenies based on BEAUti and BEAST v.1.8.3 (Drummond *et al*., 2012), with unlinked substitution models, no calibration points, a relaxed uncorrelated lognormal clock and a Yule speciation process due to a lack of fossil dates. We allowed the Markov Chain Monte Carlo (MCMC) simulation to run for 50 million generations, and then sampled a tree every 2000th generation. The effective sample size (ESS) values for each of the tree statistics showed the satisfying convergence of the Bayesian chain and adequate model mixing in the program Tracer v.1.6.0 (Rambaut, 2014). We then used TreeAnnotator v.1.8.3 (Drummond *et al*., 2012) to generate a maximum clade credibility tree with mean node heights and a 20% burn-in before ending the analysis.

2.3. Statistical analyses We performed all analyses in the statistical software R v. 3.3.1 (R Development Core Team, 2016). We used phylogenetic generalized least-squared (PGLS) regressions (Freckleton, 2002) to account for statistical non-independence of data points because of shared ancestry of species in the R package based on

the molecular phylogeny. To evaluate the phylogenetic relationship of the covariance in the residuals, we estimated the phylogenetic scaling parameter Pagel's λ of these regressions using maximum-likelihood methods. We used likelihood ratio tests to establish whether the models with the maximum-likelihood value of λ differed from models with values of $\lambda = 0$ or $\lambda = 1$, respectively. λ close to 0 indicated phylogenetic independence and λ close to 1 indicated a strong phylogenetic association of the traits. Since digestive tract was subject to a wide range of selective pressures that acted simultaneously, we assessed the relationships between digestive tract and ecological type based on Markov Chain Monte Carlo GLMMs, implemented in the MCMCglmm R package v2.20 (Hadfield, 2010) with body size added as a covariate to account for allometric effects. In all cases, we used inverse-Wishart priors ($V = 1$, $v = 0.002$). Each model was run for 5 100 000 iterations with a 100 000 burn-in and a thinning interval of 5000. After running the models, we examined the autocorrelation of samples to make sure that it was < 0.1. We presented parameter estimates from models as the posterior mode and the 95% lower and upper credible intervals (CIs) of the posterior samples. Significance values (pMCMC) were the proportion of samples from all the iterations that are greater or less than 0. We used PGLS treating digestive tract length as the response variable, altitude and latitude as the predicted variables, and body size and sample size as covariates to test the effects of altitude and latitude on digestive tract length. In the mean time, we tested the effects of temperature and precipitation on digestive tract length.

3. Results

MCMCglmm revealed that ecological type significantly affected variation in the digestive tract length across 35 species of anurans (Table 1). Digestive tract length was positively correlated with body size (Table 1). Aquatic and terrestrial species had relatively larger digestive tract than arboreal species after controlling body size effect (Figure 1). When ecological type could be split into simply 'terrestrial' (for terrestrial and arboreal) and 'aquatic' (for aquatic and semi-aquatic), we found that terrestrial species tended to have relatively longer digestive tract than aquatic species (Post. mean = 0.064, CI 95% = –0.050–0.175, P = 0.253).

PGLS revealed that interspecific variation in digestive tract length was positively correlated with latitude, but not altitude (Table 2). Meanwhile, average monthly
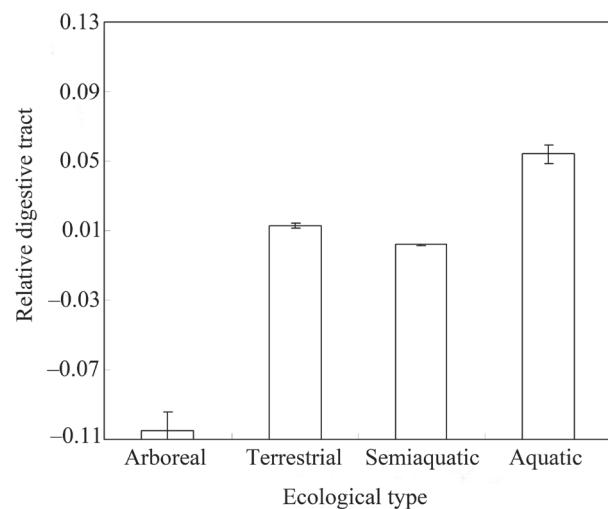
Figure 1 The difference in digestive tract length among ecological types among 35 species of anurans. Relative digestive tract size was estimated using the residuals from observed digestive tract length minus predicted digestive tract length based on the regression of digestive tract length on body size when controlling body size effect.

monthly temperature and precipitation was not correlated with variation in relative digestive tract length (Table 2).

4. Discussion

Our study demonstrates a marked effect of ecological type on relative digestive tract size across 35 species of anurans. The digestive tract length is positively correlated with latitude, but not altitude, average monthly temperature and precipitation. Below we discuss what may underlie the ecological and geographical reasons for the variation of digestive tract length in anurans.

Differences in environmental conditions was related to variations in organ morphology in organisms (Hammond *et al.*, 1999; Zhong *et al.*, 2017; Liao *et al.*, 2011; Liao *et al.*, 2013; Pascoal *et al.*, 2017; Martinez *et al.*, 2018; Møller *et al.*, 2018; Samuk *et al.*, 2018). In particular, variation in digestive tract length associated with food changes can adapt different environmental types (Hammond *et al.*, 1999). A comparison for digestive tract between individuals from different ecological types is important for understanding how physiological traits are affected by environmental conditions, and consequently, how they evolve (Chown and Nicolson, 2004). Actually, environmental difference often determines food availability; in frogs mostly via affecting the abundance of digestible foods (Wen and Zhang, 2010; Shi *et al.*, 2011). In this study, we found that ecological types significantly affected the digestive tract length, with arboreal species exhibiting shorter digestive tract than aquatic species.

Table 1 The effect of ecological type on digestive tract length when controlling body size among 35 species of anurans using MCMCglmm.

Predictors	Digestive tract length			
	Post. mean	l-95% CI	u-95% CI	P
Ecological type	0.06	0.01	0.112	0.022
Body size	1.012	0.704	1.323	<0.001
Sampling size	−0.011	−0.018	−0.005	<0.001

Table 2 The relationships between digestive tract length and altitude and latitude, as well as temperature and precipitation when controlling body size among 35 species of anurans using PGLS. Phylogenetic scaling parameters (superscripts following λ denote *P*-values of likelihood ratio tests against models with λ = 0 and λ = 1, respectively).

Predictors	Digestive tract length			
	λ	β	t	P
Latitude	<0.01[1, <0.01]	<0.001	2.27	0.031
Altitude		<0.001	−0.144	0.887
Body size		1.022	6.887	<0.001
Sampling size		<0.001	−3.214	<0.001
Temperature	0.40[0.644, <0.01]	<0.001	0.352	0.727
Precipitation		<0.001	-0.5	0.623
Body size		1.102	6.745	<0.001
Sampling size		<0.001	−3.158	<0.001

Often individuals foraging more indigestible materials result in increasing area and length of digestive tract to digest and absorb more nutrients (Penry and Jumars, 1987; Sassi *et al.*, 2007). For anurans, there are evidences that individuals consuming higher proportion of indigestible materials (e.g., high-fiber food) have larger intestines than individuals consuming arthropods (Naya *et al.*, 2009). Hence, we inferred that arboreal species probably consumed more digestible materials than aquatic species, which can explain consequently the effect of ecological type on variation in relatively digestive tract length among species.

A positive correlation between digestive tract length and body size has been reported in most anurans species (Naya *et al.*, 2009; Lou *et al.*, 2013; Ma *et al.*, 2016; Wang *et al.*, 2017). Energy requirements can lead to the variation in digestive tract per unit body mass (Pulliainen, 1976). Hence, larger species should need more energy than smaller species, and thus possessing longer digestive tracts (Lou *et al.*, 2013). We found that there was positive relationship between digestive tract length and body size, suggesting that longer digestive tract evolution for larger species was possible to correlate with energy requirements.

Previous studies have shown that individuals living in lower altitude/latitude with higher temperature and less precipitation possess relatively shorter guts than those living in higher altitude/latitude with lower temperature due to more digestible materials (Naya *et al.*, 2009; Lou *et al.*, 2013; Ma *et al.*, 2016; Wang *et al.*, 2017). In particular, increased composition of the digestible foods

(e.g., animal-based foods) and the decreased indigestible foods (e.g., low-quality, high-fiber food) in low altitude may result in decreasing digestive tract length among populations in frogs (Naya *et al.*, 2009; Ma *et al.*, 2016). Here, we did not find an increase in digestive tract length with increased altitude. In the contrast, our findings of the latitudinal increase in digestive tract length are consistent with the three frog species examined for altitudinal variation in the digestive tract (Naya *et al.*, 2009; Lou *et al.*, 2013; Wang *et al.*, 2017). Hence, more digestible materials in low latitude can promote short digestive tract length in anurans. Moreover, we found that variation in digestive tract length was not correlated with both temperature and precipitation across 35 species. Future study need collect more species to address these relationships.

Taken together, differences in environmental conditions shape variation in digestive tract length to meet differential selective forces or constraints. We observe significant effect of ecological type on digestive tract length, terrestrial species tending to have relatively longer digestive tract than aquatic species among 35 species of anurans. Although digestive tract length is positively correlated with latitude, it is not correlated with altitude, temperature and precipitation.

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Appendix

Table S1 Species, latitude (°), altitude (m), temperature (°C), precipitation (mm), sample size, body size (mm), digestive tract length (mm) and ecological type.

Species	Latitude	Altitude	Temperature	Precipitation	Samplings	Body size	Digestive tract length	Ecological type
Amolops lifanensis	30.88	2650	8.04	932	4	51.44	124.75	Aquatic
Amolops loloensis	32.05	1745	10.71	563	3	56.15	148.55	Terrestrial
Amolops mantzorum	30.55	1700	11.63	1080	15	51.32	87.25	Aquatic
Bombina maxima	27.7	2685	10.33	861	10	58	77.92	Semiaquatic
Bufo andrewsi	30.55	1700	11.63	1080	18	75.92	103.78	Terrestrial
Bufo gargarizans	30.8	290	19.21	1296	7	106.07	262.67	Terrestrial
Bufo melanostictus	27.38	780	14.92	1123	10	84.6	110.2	Terrestrial
Bufo minshanicus	32.05	1745	10.71	563	3	67.49	140.39	Terrestrial
Bufo tibetanus	30.52	3500	0.1	616	3	57.86	159.53	Terrestrial
Feirana quadranus	106.55	1674	11.63	1160	10	69	201.28	Aquatic
Fejervarya multistriata	28.62	265	19.33	582	20	38.54	56.44	Semiaquatic
Hyla annectans jingdongensis	27.53	2028	6.67	1071	17	33.59	36.06	Arboreal
Hyla tsinlingensis	29.1	1749	9.96	1510	3	32.1	64.3	Arboreal
Hylarana daunchina	27.98	640	14.92	943	3	43.7	87.68	Semiaquatic
Hylarana guentheri	30.83	338	18.75	551	13	64.28	102.95	Semiaquatic
Kaloula rugifera	32.3	1400	11.63	1166	3	36.65	76.55	Terrestrial
Kaloula verrucosa	26.88	1834	14.75	1016	14	38.5	54.95	Terrestrial
Microhyla fissipes	30.08	280	19.25	686	13	18.57	46.26	Semiaquatic
Nanorana ventripunctata	27.55	2028	6.67	1071	3	35.49	49.36	Semiaquatic
Odorrana grahami	27.27	1860	12.38	885	17	66.23	87.28	Aquatic
Odorrana hejiangensis	28.62	747	16.13	1185	4	46.87	76.53	Aquatic
Odorrana margaretae	32.67	1046	14.25	1424	3	70.26	97.39	Aquatic
Odorrana schmackeri	32.13	966	16	1235	23	44.42	66.52	Aquatic
Paa boulengeri	30.88	2650	6.29	932	3	70.38	169.96	Aquatic
Paa yunnanensis	28.65	1350	14.04	1281	5	61.92	187.94	Aquatic
Pelophylax hubeiensis	30.22	250	10.72	980	12	36.72	45.93	Semiaquatic
Pelophylax nigromaculata	30.83	338	18.54	551	4	53.88	143.5	Semiaquatic
Pelophylax pleuraden	27.18	1413	16.83	885	12	47.31	69.59	Semiaquatic
Polypedates megacephalus	29.83	688	16.79	708	16	42.74	46.77	Arboreal
Rana chaochiaoensis	27.17	1935	11.5	885	10	46.67	63.33	Semiaquatic
Rana kukunoris	31.5	3543	-2.08	1088	4	49.7	72.88	Terrestrial
Rana omeimontis	28.78	281	19.13	378	8	50.25	52.4	Terrestrial
Rhacophorus chenfui	28.3	380	19.08	1203	3	38.58	53.68	Arboreal
Rhacophorus dugritei	28.92	2548	9.5	1524	12	41.72	53.04	Arboreal
Rhacophorus omeimontis	30.55	1700	11.63	1080	11	59.22	61.74	Arboreal

Table S2 Genbank accession numbers for the gene sequences used to generate the phylogeny.

| Species	12S	16S	CYTB	RAG1	RHOD	TYR
Amolops lifanensis	DQ359981.1	DQ204482.1	KJ008458.1		DQ360034.1	DQ360065.1
Amolops loloensis	AB211455.1	AB211478.1	KJ008431.1		DQ360008.1	DQ360039.1
Amolops mantzorum	DQ359970.1		KJ008405.1	EF088240.1	DQ360023.1	DQ360054.1
Bombina maxima	DQ925758.1	DQ925780.1	EU531274.1			
Bufo andrewsi	AF160764.1	AF160782.1		DQ158353.1	DQ283905.1	
Bufo gargarizans	NC_008410.1	NC_008410.1	JN647482.1	KF666177.1		
Bufo melanostictus	AY458592.1	AB167927.1	AF249082.1	KT031693.1	AF249097.1	
Bufo minshanicus	KM587710.1	KM587710.1				
Bufo tibetanus	AF160766.1	AF160784.1	AF171193.1			
Feirana quadranus	GQ225906.1	GQ225932.1	KX021999.1	HM163591.1	EU979886.1	EU979981.1
Fejervarya multistriata		AF206466.1	AB296096.1	AB526660.1	DQ458271.1	EU980027.1
Hyla annectans jingdongensis	KP742564.1		AY843821.1		AY844574.1	AY844045.1
Hyla tsinlingensis	KP742646.1	KP212702.1	JX870448.1			
Hylarana daunchina	KU840524.1	KU840597.1	KF020631.1	KU840723.1		KU840782.1
Hylarana guentheri		KF185060.1	KR264131.1	KR264365.1	DQ284009.1	KR264440.1
Kaloula rugifera	JX678894.1	JX678911.1	KT878719.1			
Kaloula verrucosa	KC822507.1	KC822507.1				
Microhyla fissipes	AB201177.1	DQ512876.1	AB201223.1	AY364198.1	AY364383.1	KC180221.1
Nanorana ventripunctata	DQ118457.1	EU979839.1		HM163585.1	EU979866.1	EU979959.1
Odorrana grahami	EF453731.1	EU861555.1		EF088257.1	DQ360016.1	DQ360047.1
Odorrana hejiangensis	KU840531.1	DQ360006.1		KU840727.1	KU840683.1	KU840788.1
Odorrana margaretae	DQ359964.1	EU861566.1	KJ815050.1	EF088261.1	DQ360017.1	DQ360048.1
Odorrana schmackeri	KU840532.1	KU840590.1	KJ149452.1	KU840726.1	DQ360020.1	DQ360051.1
Paa boulengeri	EU979791.1	EU979851.1	JX676597.1	HM163604.1	EU979918.1	EU980033.1
Paa yunnanensis	GQ225869.1	GQ225873.1	KF199150.1	HM163593.1	DQ458263.1	EU979976.1
Pelophylax hubeiensis	AF205547.1	AF315137.1				
Pelophylax nigromaculata	DQ359961.1	JQ621942.1	DQ006266.1	AB360184.1	DQ283838.1	DQ360045.1
Pelophylax pleuraden	JN541324.1	JQ621943.1	KR264150.1	KR264384.1	DQ360011.1	DQ360042.1
Polypedates megacephalus	KU840483.1	AY880519.1	AB451722.1	EU924517.1	EU924545.1	KC180271.1
Rana chaochiaoensis	DQ359975.1	DQ289107.1		KX269557.1	DQ360028.1	DQ360059.1
Rana kukunoris	KX269185.1	KX269185.1	JX486345.1	GQ285780.1	GQ285798.1	GQ285816.1
Rana omeimontis	KX269193.1	DQ289108.1	AF274928.1	KX269558.1		KX269785.1
Rhacophorus chenfui	GQ204763.1	KU840563.1	EU924603.1	EU924519.1	EU924547.1	KU840751.1
Rhacophorus dugritei	EF564471.1	EF564541.1	EU924605.1	GQ285768.1	EU215571.1	EU215601.1
Rhacophorus omeimontis	KU840492.1	KU840564.1	EU924612.1	EU924528.1	EU215565.1	KU840753.1
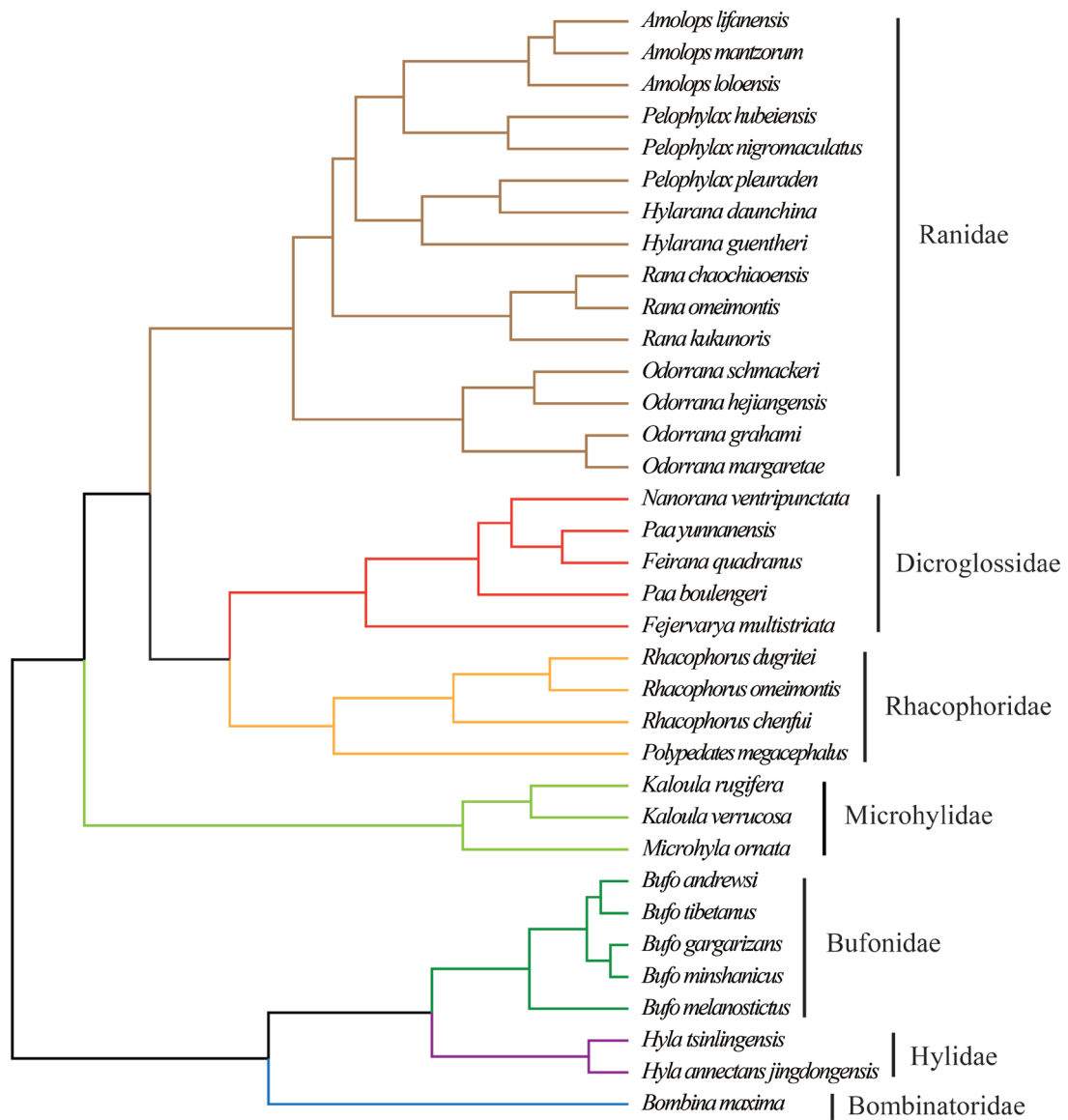
Figure S1 The phylogenetic tree of the 35 species of anurans in the comparative analysis.